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5-Hydroxymethylcytosine (5-hmC) Monoclonal Antibody [HMC/4D9]

(Catalog # A-1018)

Background
5-hydroxymethylcytosine (5-hmC), as a sixth DNA base with functions in transcription regulation, has been detected to be abundant in human and mouse brain and embryonic stem (ES) cells. In mammals, it can be generated by oxidation of **5-methylcytosine** (5-mC), a reaction mediated by the ten-eleven translocation (TET) family of 5-mC hydroxylases. 5-hmC was demonstrated to play an important and different role than 5-mC in the regulation of DNA methylation, chromatin remodeling, and gene expression.

Concentration
1 mg/ml

Description
Mouse monoclonal antibody to 5-Hydroxymethylcytosine (5-hmC), clone HMC/4D9

Specificity
Modified base 5-hydroxymethylcytosine (5-hmC), a broad range of species

Isotype
IgG1

Formulation
10 mM phosphate buffer, 150 mM NaCl, pH 7.4

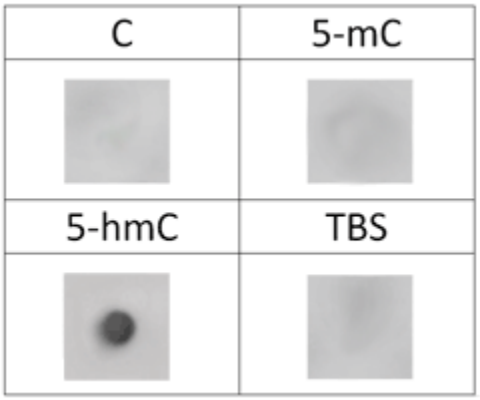
Storage
4°C. For long-term storage, aliquot and store at -20°C. Avoid repeated freezing and thawing. Multiple freeze/thaw cycles may result in decreased performance. Stable for 6 months from the date of shipment.

Purity
Protein A purified

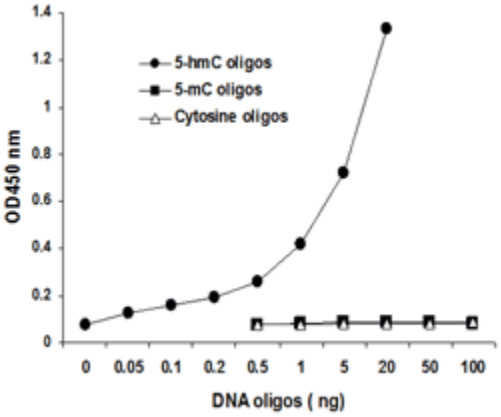
Handling Recommendations
For maximum recovery of the products, centrifuge the vial prior to opening the cap

Alternative Names
5-hydroxymethylcytidine, anti-5-hydroxymethylcytidine, anti-5-hydroxymethylcytosine, anti-5hmC, anti-5-hmC, anti-5-hmeC, anti-5hmeC, 5hmC, 5hmeC, 5-hmeC

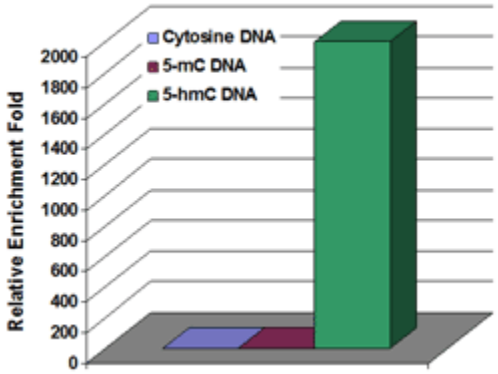
Application
Immunofluorescence: 1:200 – 1: 500, Immunohistochemistry: 1:200 – 1: 500, ELISA: 1:1000 – 1:2000, Dot Blot: 1:2000, hMeDIP: 0.5-1 µg/10^6 Cells



Dot blot analysis of A-1018 with DNA oligos containing cytosine (C), 5-methylcytosine (5mC), or 5-hydroxymethylcytosine (5hmC). 10 ng of C, 5mC, and 5hmC DNA oligos each were spotted on a membrane. The membrane was incubated with 0.5 µg/ml of Epigentek's 5hmC Clone HMC/4D9 antibody (1:2000 dilution), then with peroxidase conjugated goat anti-mouse IgG (dilution 1:3000). Specific signal was only observed with 5-hmC DNA oligos. Fig. 2. Demonstration of high sensitivity and specificity of Epigentek's 5-hmC Clone HMC/4D9 antibody against 5-hmC DNA. Synthetic DNA oligos containing 5-hmC, 5-mC or only cytosine, respectively were added into the assay wells at different concentrations and then specific signals was only detected with 5-hmC DNA.



Demonstration of high sensitivity and specificity of Epigentek's 5-hmC Clone HMC/4D9 antibody against 5-hmC DNA. Synthetic DNA oligos containing 5-hmC, 5-mC or only cytosine, respectively were added into the assay wells at different concentrations and then specific signals was only detected with 5-hmC DNA.



Selective enrichment of hydroxymethyated DNA. 50 pg of unmethylated, methylated, and hydroxymethylated DNA oligos were each spiked into fragmented human genomic DNA (500 ng). hMeDIP was processed with Epigentek's 5-hmC Clone HMC/4D9 antibody. Eluted DNA was analyzed by real time PCR with the primers to detect the presence of spiked DNA oligos. Fold-enrichment represents the amount of recovered DNA oligos and was calculated based on the Cts.